Analysis of Adenosine Triphosphate in Spatially Distributed Planetary Analog Field Samples to Inform Biosignature Detection Missions

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By

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SUMMARY

New discoveries of potentially habitable environments elsewhere in our solar system, and at the extremes here on Earth, have reopened the imagination to possibilities for extraterrestrial life. Planetary field analog research enables us to study the impact of similar extreme environmental stressors and the bioactivity of an ecosystem. This thesis research was designed to better understand biosignature detection in extreme environments by exploring distributions and patterns of biosignatures in harsh planetary environments. Adenosine triphosphate (ATP) was used as a proxy of bioactivity due to its ubiquitous role in terrestrial metabolism and can be quantified easily by a bioluminescence assay. Observing variations in concentrations of ATP can provide insight on where bioactivity becomes concentrated, or evenly distributed which is essential in the search for life outside of Earth. A variety of chemical and physical studies of samples from analog locations aids in understanding the limits of life terrestrially, and therefore can help make more informed predictions about the potential habitability on other planetary bodies.

CHAPTER 1

1. INTRODUCTION

From the beginning of civilization, mankind has often wondered whether or not we are alone in this universe, and if life does exist elsewhere, what does that life look like. In the past few decades, there has been promising new discoveries pointing towards potentially habitable environments elsewhere in our solar system and at the extremes here on Earth (Kieft, 2016). Advances in remote detection technologies have enabled scientists to better observe the surface of planetesimal bodies in our solar system (Cable et al., 2020), while new breakthroughs in microbiology show organisms adapting to many extreme conditions (Kieft, 2016 ; Merino et al., 2019). New discoveries of terrestrial forms of life found in environments previously thought to be uninhabitable have reopened the imagination to possibilities for extraterrestrial life (Amils et al., 2007), now that life on Earth is much more robust than originally considered. Celestial bodies including Mars, Europa, and Enceladus are important astrobiological targets with chemistries and physical conditions similar to locations found here on Earth (Davila & McKay, 2014), and thus the search for extant life can be informed further through studies of those similar terrestrial locations, or planetary analogs.

1.1. PLANETARY LIFE DETECTION

The search for life began on Mars when NASA's Viking Lander began its suite of onboard biological experiments in 1976, but results proved to be ambiguous for a number of reasons. The presence of heavy oxidants in the regolith caused ambiguities in the GC-MS data as well as cosmic radiation likely degrading the unprotected potential organics (Bell, 2008) on the surface. More recently, however, Mars Opportunity discovered mineralogical evidence of ancient liquid water (Andrews-Hanna et al., 2007) and the Phoenix Lander directly observed water ice (Zorzano et al., 2009) while further observations from orbit reveal ancient oceans, river systems, and deltas (Amador & Ehlmann, 2020). Scientific evidence further grew with MSL's discovery of organics in the mudstone of Gale crater and a history rich with hypersaline and acidic fluids (Pontefract et al., 2017; Tosca et al., 2008). NASA's upcoming Mars 2020 mission is designed to address key questions on the possibility for ancient Martian life by studying the history of

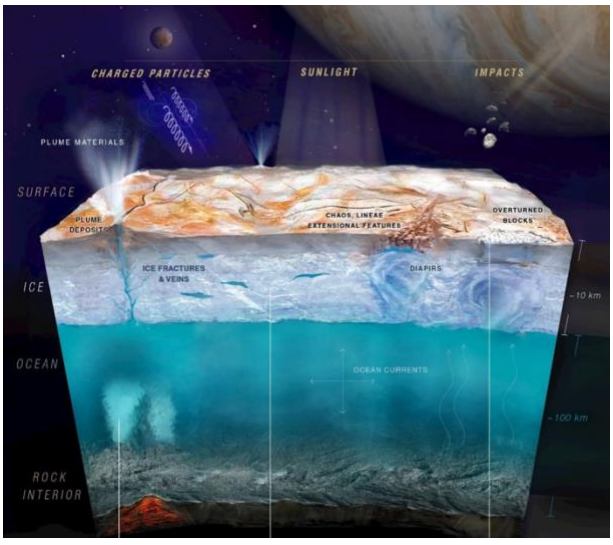


Figure 1.1. Diagram of Europan crust (Europa Lander Science Definition Team Report).

the red planet's climate and searching for signs of biosignatures in the geological record. The Perseverance rover, for the first time in history, will have the ability to collect 43 samples and cache them on the surface for future return to Earth. With so few opportunities to collect Martian regolith and rock cores, unique sampling techniques and multi-level *in situ* experiments were employed to inform the down-selection of sampling that maximizes scientific output.

1.2. ASTROBIOLOGICAL TARGETS

Located much further away in the outer solar-system, icy satellites of Jupiter and Saturn are some of the most important astrobiological targets and, unlike Mars, could potentially be able to host life today. Europa, for instance, is an intriguing moon that orbits the gas giant Jupiter. It is thought to host a liquid subsurface ocean in direct contact with a rocky, silicate, ocean floor (Kivelson et al., 2000; Zimmer, et al., 2000), and thus likely possess geothermal sources of energy (Chyba & Hand, 2001). Galileo NIMS data of Europa's surface suggest the presence of sulfates, which has fueled speculation of a salt-rich ocean (Brown & Hand, 2013). Further investigations of Europa's surface using the Hubble Space Telescope, irradiated sodium chloride (Thomas et al., 2017) was detected in the chaos terrain and is therefore indicative of endogenous sodium chloride. The global ocean is then covered in a thick ice crust, ~100-150 km (Kivelson et al., 2000), which could serve as a radiation shield to any underlying organic material. It is believed that Enceladus also hosts a subsurface liquid ocean (Hemingway & Mittal, 2019) and evidence shows that an enormous plume at its south pole is spewing small molecules like H_2, CH_4, NH_3 (Magee

& Waite, 2017), and potentially even macromolecules (Postberg et al., 2018) thousands of kilometers into space, based on CASSINI/NIMS data.

1.3. PLANETARY ANALOG RESEARCH

Field analog research in astrobiology enables us to learn more about what life in similar extreme environments might be like (Martins et al., 2015 ; Horneck et al., 2016). Studying samples from field sites with characteristics that resemble planetary bodies in our solar system can helps address gaps in our understanding of habitability and biosignature detection (Lorek et al., 2015). Field sites selected in this research range in representation of contemporary, remnant, and relict environments and their ecosystem. This is important for understanding how life co-evolves with its environment to produce detectable signals of a past or present living world**.** Currently, the symbiotic relationship and evolution of bioactivity and its extreme ecosystem are not well understood (Lorek et al., 2015 ; Martins et al., 2017). A variety of chemical and physical studies of samples from analog locations aids in the quest to understanding the limits of life terrestrially, and therefore make more informed predictions about the potential habitability on other planetary bodies.

1.3.1. Icelandic Analog

Historically, Iceland has been used as a Mars analog due to its recent volcanic eruptions providing a fresh landscape, low anthropogenic contamination, wind-blown alluvial planes and glaciated fields (Cockell et al., 2011 ; Cousins & Crawford, 2011 ; Bagshaw et al., 2011). Sandy planes of volcanic deposits located in the highland regions are low in bio abundance and provide excellent sites for sample heterogeneity exploration (Gentry et al., 2015). The chemical and geologic diversity that develops in this environment

is a good representation of the diversity in surfaces in which ancient lifeforms would have evolved, adapted, and been preserved (Rader & Simpson et al., 2020).

1.3.2. Hypersaline Lakes

Hypersaline lakes are equipped with relatively low water activity, can demonstrate extreme pH levels, and high osmotic pressures, causing certain stress on microorganisms that are present (Pontefract et al., 2017). Salt-rich environments, with varying concentrations of NaCl, $MgSO_4$, perchlorates and various other sulfates (Brown & Hand, 2013; Vance, Bouffard, Choukroun, & Sotin, 2014), possess unique chemistry tied to varying temperatures and pressures, and thus serve as analogs for ancient oceans on Mars or potentially present day brines on Mars, Europa, or Enceladus.

1.4. ATP AS A TERRESTRIAL BIOMARKER

Adenosine triphosphate (ATP) is a proxy of bioactivity due to its ubiquitous role in terrestrial metabolism and can be quantified easily by a bioluminescence assay, but is an

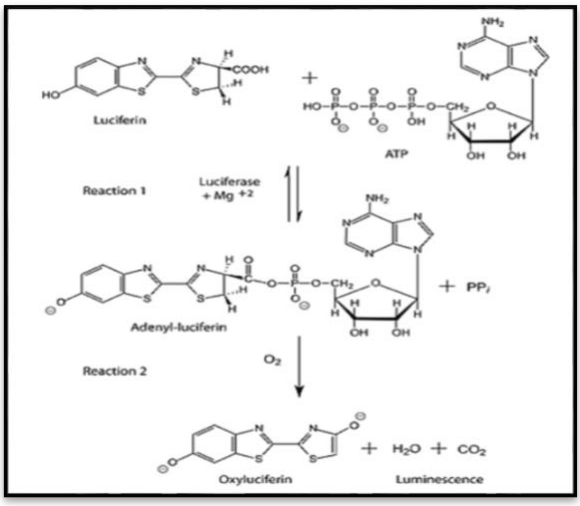


Figure 1.2. Reaction pathway for the Bioluminescence Luciferase ATP assay.

evolved molecule likely specific to terrestrial life. Historically, measurements of ATP have been used as a valuable means to estimate the biomass of viable microbes (Tuovila et al., 1987) because ATP is rapidly hydrolyzed upon the death of a microbial cell and does not stay associated with cell remains in most environments (Holm-Hansen, 1973). A simple and effective ATP assay using external standards and luminescence detection has previously been developed and can provide ppb limits of detection (Gentry et al., 2015). The luciferase enzyme, commonly found in fireflies, converts the compound luciferin into the excited state of oxyluciferin in the presence of ATP, which luminesce upon decay to the ground state (Holm-Hansen, 1973).

The investigation of ATP, used as proxy for terrestrial bioactivity, in harsh, seemingly lifeless environments is demonstrated in this thesis research to better understand biosignature detection on other planetary bodies. Distribution or concentration patterns of ATP from seemingly homogenous environments are reported to help understand sample diversity for future sample collection missions on other celestial bodies. Additionaly, the bioluminescent analysis of ATP in extreme saline and low water activity conditions are explored to understand preservation of life on past or present ocean worlds.

CHAPTER 2

2. Spatial Distributions of Adenosine Triphosphate in Icelandic Volcanic Regions as an Analog of Mars Missions.

2.1. Introduction

Partially in an attempt to discover signs of life on Mars, NASA's 2020 mission will be the first exploration in which Martian rocks and soil will be collected and stored for a future return. The belly of the rover (Figure 2.1) houses a large robotic arm that contains the rotating drill carousel that will be used to collect various samples into 43 tubes (NASA 2020 Mission – Perseverance Rover). With a limited number of samples to be cached on the surface, Martian analog field studies are extremely useful for understanding sampling

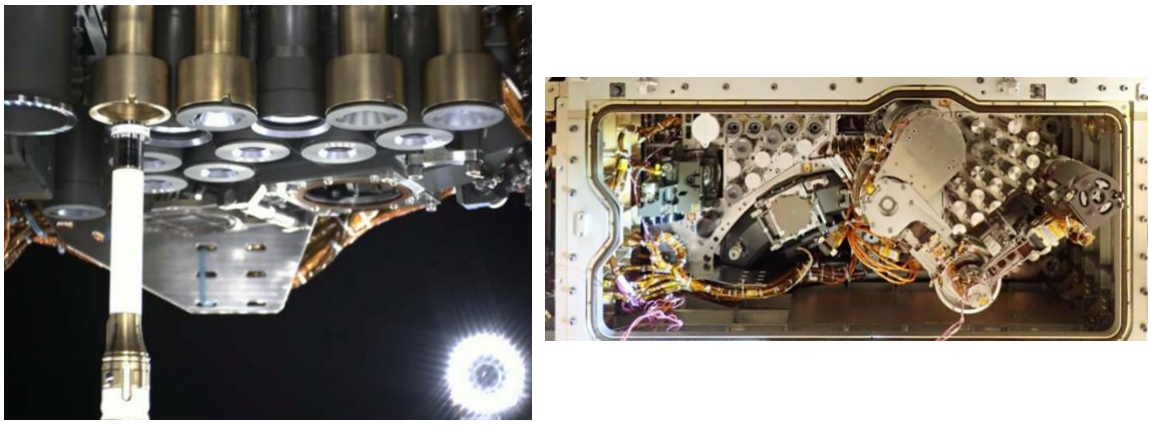


Figure 2.1. NASA JPL, Perseverance rover – The Adaptive Caching Assembly has 43 sample tubes for caching samples (Image accessed online. www.nasa.mars.gov).

strategies critical for ensuring that the samples with the greatest chances of harboring signals of life be collected for later return missions.

2.1.1. Analog Selection

Iceland is considered an analogue for Mars due to its cold temperatures, volcanic regions, and minimal anthropogenic contamination (Cockell et al., 2011). The sub-glacial volcanism in Iceland in particular serves as an analog for the creation of habitable environments in middle Mars era (Preston & Dartnell, 2014), while the basaltic plains and barren deserts are analogs for modern day Mars (Allen, et al. 1981). Dyngjusandur (Table 2.1) is an alluvial plain where the sediment is mechanically redistributed on a regular basis, which is the likely cause of its continued barrenness, and thus serves as an analogue for basaltic alluvial plains on Mars.

Icelandic lava fields are important in astrobiology due to the extreme environmental conditions that make it difficult for life to thrive (Figure 2.2). This includes low nutrient availability, desiccation, temperature extremes, fresh landscape, and due to the isolated locations, free of significant outside contamination or alteration. Fimmvörðuháls and Holouhraun are lava fields formed from a basaltic effusive eruption in 2010 and in 2014,

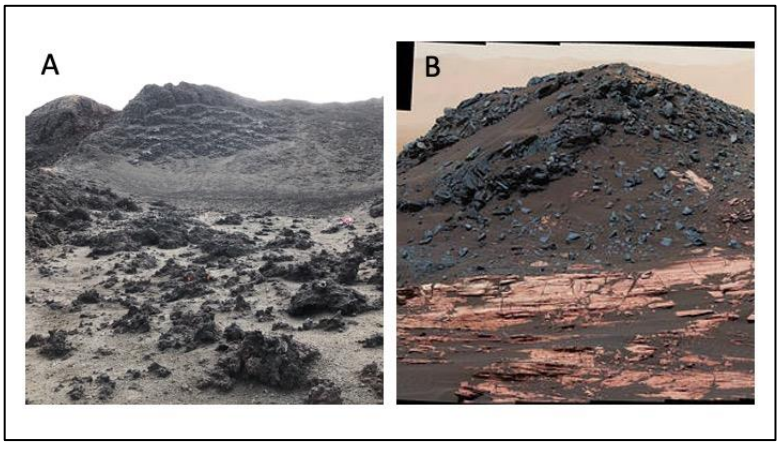
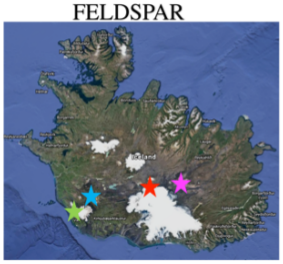


Figure 2.2. Comparison of Marian analog vs. Mars(A) Photo taken by me of Holouhraun lava field. (B) NASA photo from Curiosity rover .

respectively. Because these field sites were recently created, these lava fields also provide a means to study the rate of biological impact in a region over time.

Table 2.1. Field Site's from the 2017 and 2019 expedition.

Project	Field Site	Specific Scientific Justification
FELDSPAR 	Fimmvörduháls, Iceland (63° 38.205' N, 19° 26.820' W)	Lava field formed in April 2010 from basaltic effusive eruption, low anthropogenic contamination
	Mælifellssandur, Iceland (63° 49.000' N, 19° 10.298' W)	Recently deglaciated region of basaltic tephra, low anthropogenic contamination,
	Dygjusaunder, Iceland (64° 55.826' N, 16° 42.974' W)	Wind blown alluvial planes of volcanic tephra, low anthropogenic contamination
	Holuhraun, Iceland (64° 38.460' N, 17° 31.680' W)	Recent volcanic eruption (2014), creates new surface to conduct a temporal study.

2.1.2. Nested Triangular Sampling Scheme

Using a set of nested triangular grids (Figure 2.3), it is possible to observe how bioactivity potentially spreads or changes at distances without requiring collection of a prohibitively high number of samples. Equilateral triangles ensure that these are separated by the same distance, enabling rudimentary statistical analysis. Triangular grids are selected where surface sediment appear to be visually homogenous by color, morphology, and grain size and then are spaced from 0.1, 1, 10, to 100 meters apart, when the study

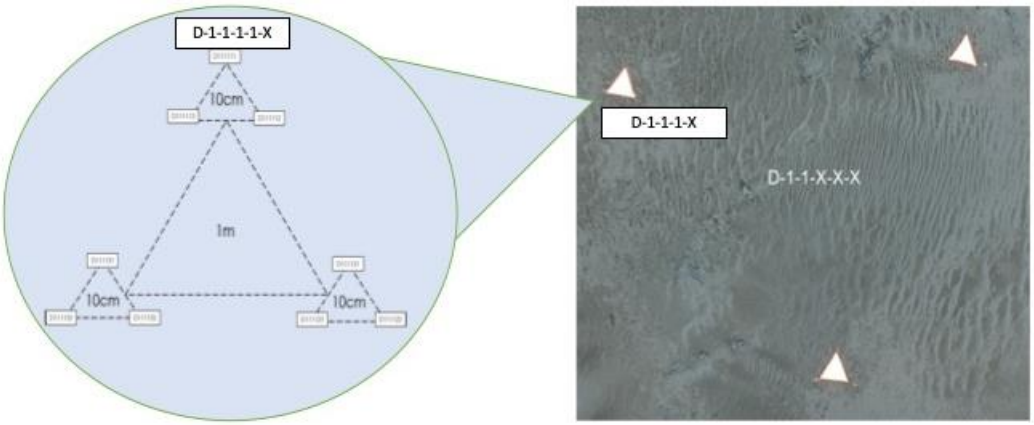


Figure 2.3. Nested grid schematic (left) and aerial image of a 10 meter grid (right).

seeks to identify spatial heterogeneity without considering these variables that often chante site-to-site (Gentry et al, 2015).

2.1.3. Adenosine Triphosphate: A Terrestrial Biomarker

While adenosine triphosphate (ATP) is an energy-storage molecule potentially not conserved across planetary environments, its ubiquity in terrestrial biology makes it attractive as a biomarker for terrestrial biota (Parnell et al, 2007). Given the relative ease of analysis via a bioluminescence assay, many terrestrial analogue studies use ATP as a proxy biomarker to understand distribution and preservation of biomarkers in planetary analogue samples. The ATP bioluminescence assay is based on the conversion of luciferin to oxyluciferin by luciferase using ATP in the presence of molecular oxygen (Chapter 1, Figure 1.1). The resulting oxyluciferin is produced in an excited electronic state, and releases a photon upon decaying to the ground electronic state. If all other reagents are in excess, ATP becomes the limiting reagent, and thus one photon per molecule of ATP in the original sample via standard first-order kinetics.

2.2. Materials and Methods

All reagents were used as obtained except where indicated. ATP Bioluminescence Assay Kits HSII were purchased from Roche Diagnostics (Sigma-Aldrich). TRIS and EDTA were obtained from Sigma-Aldrich. Stock solutions of 10M TRIS and 0.4M EDTA were prepared and used to prepare a 10X Tris-EDTA buffer solution.

2.2.1. In-Field Sample Collection

Research permits were from Vatnajokull National Park and export permits from the Icelandic Institute of Natural History. After sampling sites were identified, GPS coordinates are recorded and, if weather permitting, aerial photos of the landscape up 200 meters elevation were taken using an Inspire I quadcopter (DJI). To mitigate the risk of human contamination, each team member wore face masks, non-linting arm sleeves, gloves, and worked downwind first (Figure 2.4) never walking through the grid itself. Samples are then excavated from approximately 5 cm below the surface with a gardening shovel sterilized with isopropyl before each sample. Sterile 50 mL Falcon Tubes were used to store the collected samples and Whirl-Pak bags are used during sample preparation. During the 2019 expedition, one full 100 meter grid was collected at Dyngjusandur, and two 10 meter grids were collected at the other two locations, Fimmvörðuháls and Holouhraun.

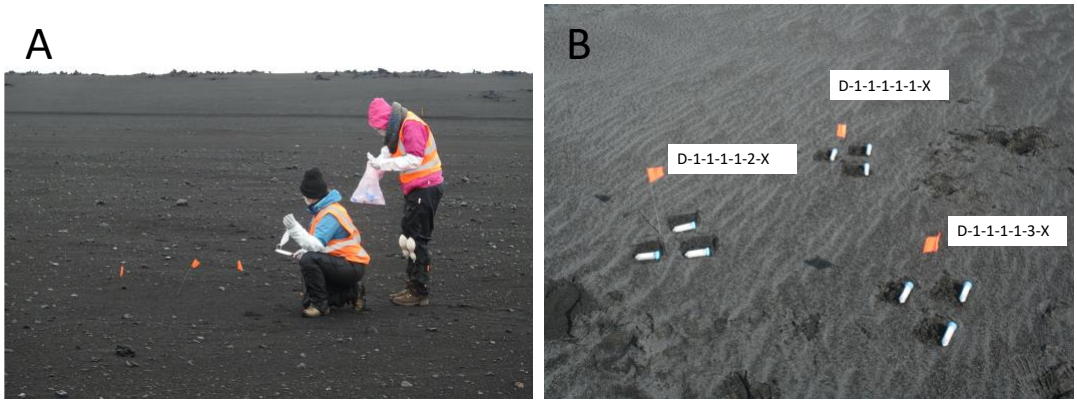


Figure 2.4. (A) Dr. Diana Gentry and Carlie Novak sampling at Dyngjusandur in 2019 and (B) collected samples from a 10 meter grid.

2.2.2. Bioluminescent Analysis of ATP

Samples were analyzed in a field lab within 48 hours after collection to reduce chances of ATP loss. The bioluminescence assay was conducted according to the Roche ATP Bioluminescence Assay Kit HS II manufacturer's instructions, with some modifications as indicated. Standard curves were generated on a daily basis. The samples were first crushed and homogenized in double layered, sterile Whirl-Pak bags using a hammer that had been covered with sterilized foil and then divided into triplicate aliquots in 2 mL centrifuge tubes. The samples were then vortexed with a Tris-EDTA buffer (1 mL 100mM TRIS, 4mM EDTA) and submerged in boiled water for 5 minutes to lyse the cells. After cooling back to room temperature, samples were centrifuged for 5 minutes. Exactly 50 µL of supernatant was then added to 50 µL of luciferase reagent immediately before analysis. Bioluminescence was then immediately measured using a Merck HY-LiTE 2 portable luminometer.

2.2.3. Moisture Content and Grain Size

Moisture content and grain size analysis took place upon return to the home lab and samples were kept frozen at -80 °C until they were used. Samples were analyzed for moisture content followed by a grain size analysis for each sample collected in 2017 from the following field sites: Fimmvörduháls, Mælifellssandur, Dyngjusaundur, and Holouhraun. Triplicate aliquots of each sample point were massed before and after a 48 hour incubation at 100 °C. Moisture content was calculated by subtracting the 'dry' mass from the 'wet' mass. After weighing the total sample, the dried sediment was passed through a set of sieves with mesh sizes of 2 mm, 0.85 mm, and 0.425 mm (Figure 2.5).

Each grain-size group was weighed and the mass was divided by the total mass to yield mass percent.

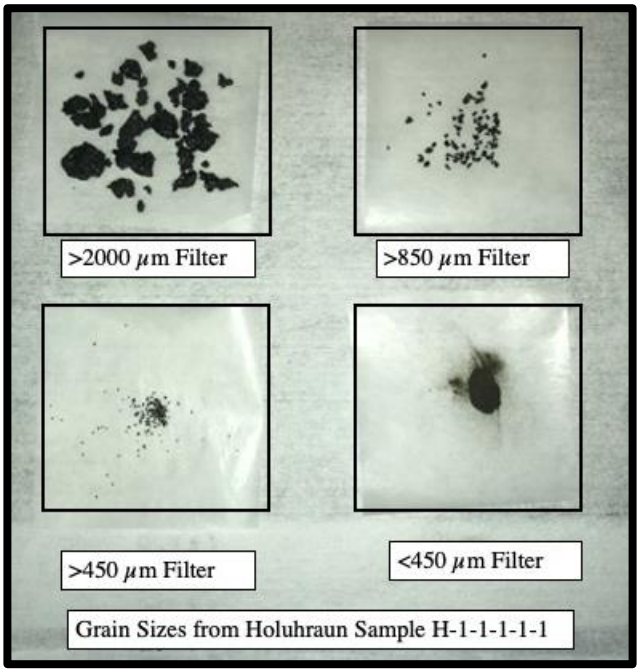


Figure 2.5. Grain sizes from one sample

2.3 Results and Discussion

Investigating distribution patterns of biomarkers and their variations in measurements on a scalar plane could significantly improve sampling strategies and biosignature detection, especially in correspondence to unmanned rover missions. Moisture content and grain size are easily measured and provide a means for comparison with ATP for understanding the biodiversity in a region. Results presented in this section for moisture content and grain size are from samples collected in 2017 but that I analyzed in 2018, as well as the ATP analysis from the 2019 expedition.

2.3.1. ATP

The bioluminescence assay has a large dynamic range scaling over several orders of magnitude of ATP concentration, with excellent repeatability and a lower limit of detection of approximately $5*10^{-12}$ M according to the standard curve. The data presented in Figure 6 and 7 show the average ATP content from each triplicate sample location at the Dyngjusandur (D - site)**,** and Holouhraun (H - sites) sites. Note that the "error bars" do not represent error inherent in the assay, but rather the spread of heterogeneity in the individual sites.

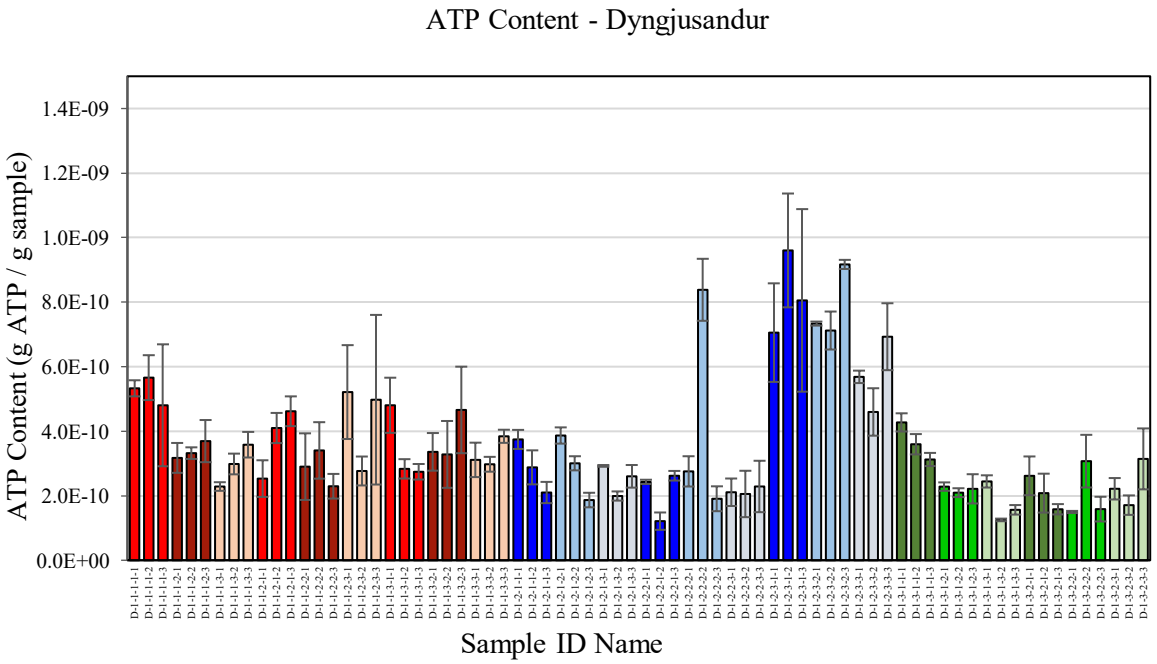


Figure 2.6. ATP Content from Dyngjusandur 2019. Each color shade group represent a 10 meter triangle. The entire graph together shows variation in ATP across one 100 meter triangle.

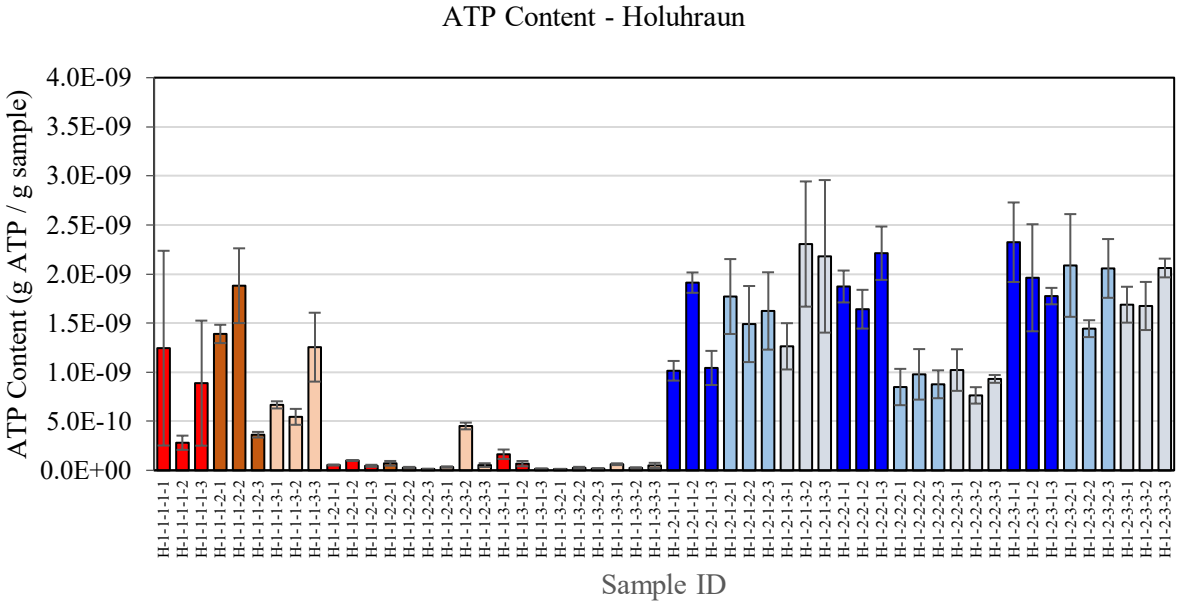


Figure 2.7. ATP Content from Holouhraun 2019. Each color shade group of represent a 10 meter triangle. Only two 10 meter grids were collected.

2.3.2. Moisture Content and Grain Size

In sediment, moisture content can vary substantially from one day to the next, and grain size correlates with which locations will retain more moisture than others (Watanabe, Matsuoka, Christiansen, & Cable, 2017) whether it has rained recently or not. The smaller

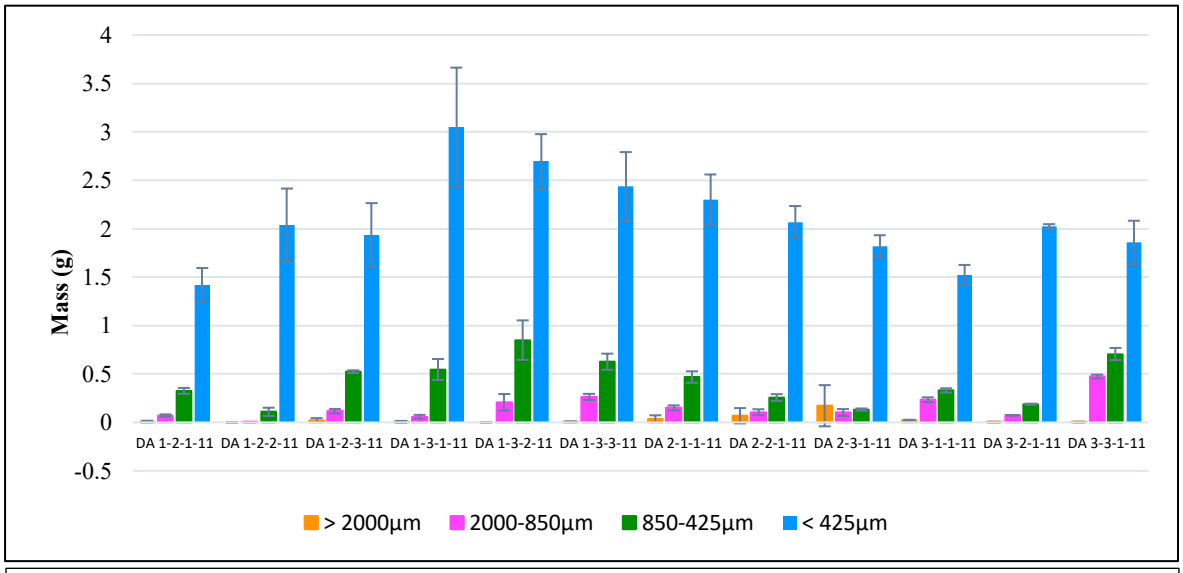


Figure 2.8. Grain size analysis for sites D-1-2-1-11 through D-3-3-1-11.

the average grain size, the larger the surface area to volume ratio, providing more surface area for water to adsorb to and therefore likely a larger habitat for microbial colonization.

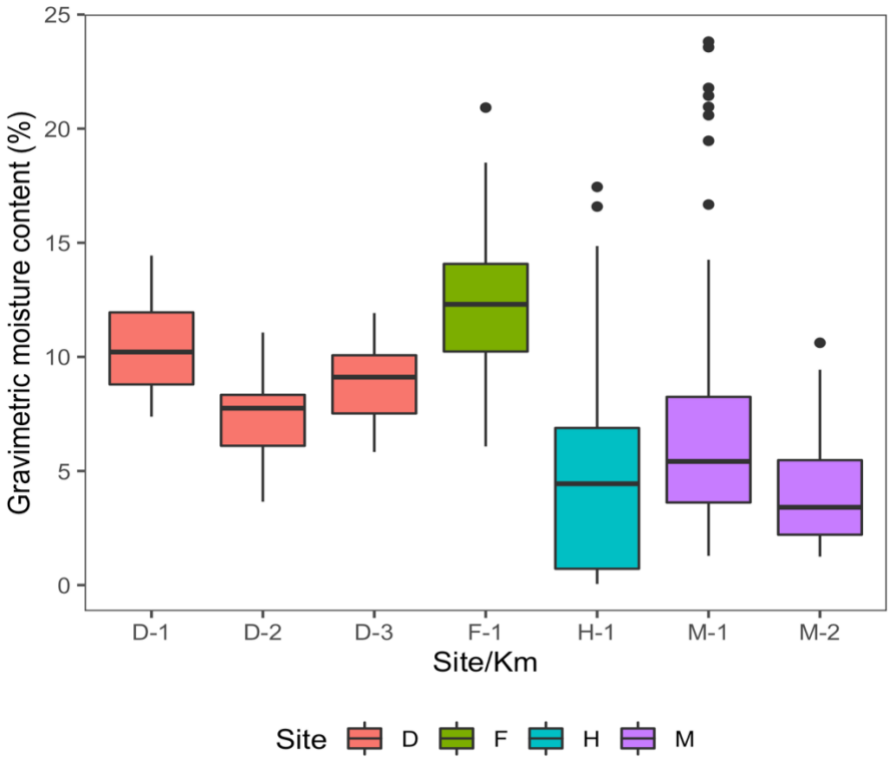


Figure 2.9. Box plot chart of average percent moisture content for each 10 cm triangle from each site

2.3.3. Conclusions

To ensure that a location has been accurately represented it is imperative to know if we have collected enough samples and the right kind of samples to maximize scientific return in future unmanned astrobiology-based missions. Observing variations in concentrations of ATP from nested grids can provide insight on where bioactivity becomes concentrated, or evenly distributed. It can be concluded that bioactivity in these regions can vary from site (Figure 2.10) and as close as 10 cm away.

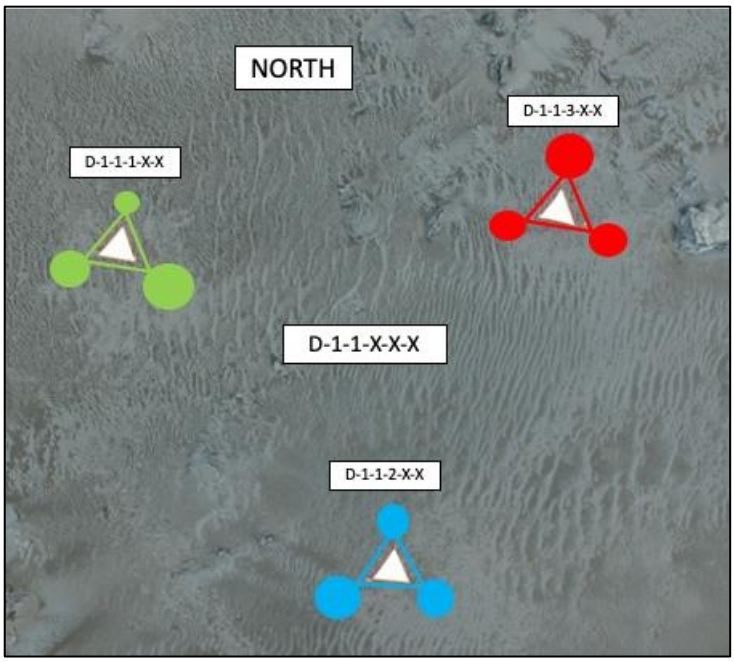


Figure 2.10. Relative ATP (g/per g sediment) concentration variations show through sized circles. Larger circles represent larger average concentrations reported from a 10 cm triangle.

Samples collected from Dyngjusandur in 2017 show interesting patterns between moisture content, ATP, and grain size (Figure 2.11). Sites with low grain size (<425 µm) had slightly higher moisture content and ATP content than those with rocky areas. This is most likely due to more surface area in the smaller grain sizes to hold onto small biomolecules. As expected, Holouhraun reported the lowest levels overall of ATP in both 2017 and 2019, where the regolith was the youngest, grain sizes and moisture content varied the most, and the conditions were the most erratic. Other factors may play a larger role in the distribution of ATP in these extreme conditions and further investigation over longer time periods are needed.

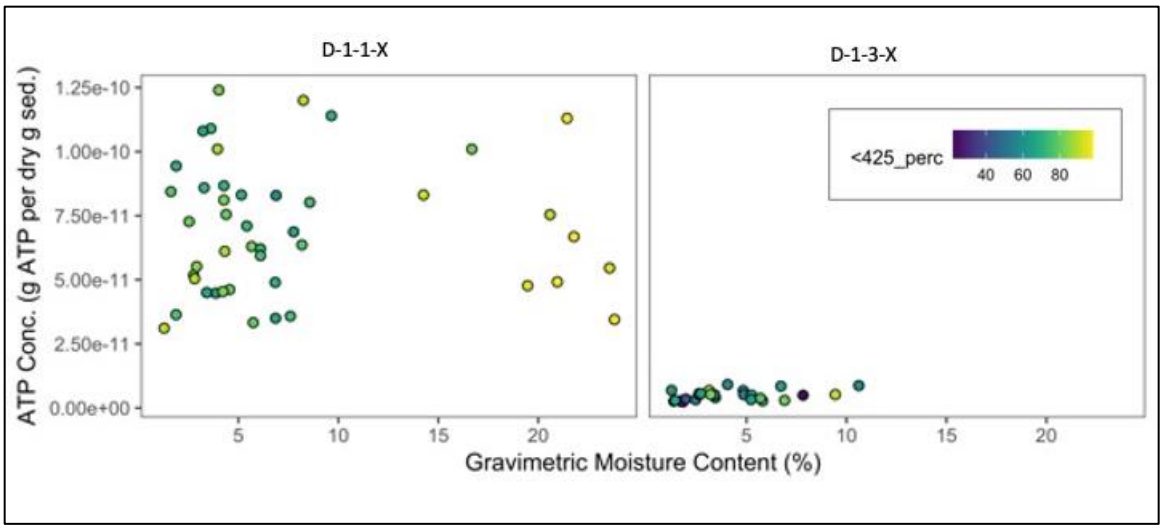


Figure 2.11. Moisture content and ATP concentration (g ATP per gram of sediment) plotted based on mass percent of sediment <425 µm from site D-1-1-X and D-1-3-X.

CHAPTER 3

3. Analysis of Adenosine Triphosphate from Hypersaline Pools using a Luciferase Bioluminescence Assay to Inform Ocean World Life Detection Missions

3.1. Introduction

There is ample evidence that Europa and Enceladus likely host a large, salt-rich, liquid ocean beneath thick ice sheets (Kivelson & Khurana, 2000), making these moons an exciting place to explore habitability in the solar system (Trumbo et al., 2020). Ground-based infrared observations (Brown & Hand, 2013) and data from the Galileo Near-Infrared Mapping Spectrometer (NIMS) of Europa's surface suggest endogenous sources of magnesium sulfate and chloride salts (Fischer & Brown, 2015). However, recent laboratory experiments of sodium chloride (NaCl) and NaCl brine evaporates under Europa-like surface conditions have demonstrated similar unique distinct spectral features discovered by NIMS (Hand and Carlson, 2015 ; Geissler et al, 1998), suggesting endogenous material may reflect a chloride-dominated composition (Trumbo et al., 2020). On Mars, there is sufficient evidence that ancient saline bodies of water (Tosca et al, 2008) have resulted in the widespread deposition of sulfate and chloride salts (Wanke et al., 2001;

Goude et al, 2016). Because these salt systems are so prevalent on important astrobiological targets, it is critical to understand how potential biosignatures produce detectable signals of past or present life, as well as how or if those biosignatures are preserved.

3.1.1. Hypersaline Pools as Ocean World Analogs

Hypersaline systems are harsh environments with salt concentrations often close to or exceeding the salt saturation point. Such high salt concentrations are toxic to most organisms because it greatly increases osmotic pressures, yet life still exists in these environments. Salt-tolerant organisms, halophiles, heavily populate hypersaline environments (Hallsworth et al 2006), with NaCl-dominated systems hosting the widest variety of microbes (van der Wielen et al, 2005) and ponds often display vivid colors of pink or blue (Figure 3.1). In contrast, $MgCl_2$-dominated brines have significantly less microbial life likely from a large drop in water available to cells (Javor, 1989).

3.1.2. South Bay Salt Works

Figure 3.1. Sodium chloride dominated ponds at South Bay Salt Works in Chula Vista, California. Photo taken by author.

Figure 3.2. Aerial image of South Bath Salt Works with collection sites numbered and color coordinated based on year collected.

South Bay Salt Works (SBSW's) is a salt factory located on the coast of the San Diego Bay in Chula Vista, California that intakes water from the Pacific ocean. The salt water is channeled through a series of ponds (Figure 3.2) and leaves behind large deposits of salt as the water evaporates and sectioned off ponds with varying intensities of NaCl and $MgCl_2$ brines. The range of salinities and conditions of water, salt, and sediment from pond-to-pond is ideal for studying the limits of life displayed across gradients of environmental stress. SBSW's is an excellent location to study where life can or cannot exist, for the purpose of ocean world life detection missions.

3.1.3. Scope of Work

While adenosine triphosphate (ATP) is an energy-storage molecule potentially not conserved across planetary environments, its ubiquity in terrestrial biology makes it attractive as a biomarker for terrestrial biota (Parnell et al, 2007). Given the relative ease of analysis via a bioluminescence assay, many terrestrial analogue studies use ATP as a proxy biomarker to understand distribution and preservation of biomarkers in planetary analogue samples (Radar et al, 2020 ; Gentry et al, 2015). However, ATP analysis of high salt content brines is not well understood, especially when dealing with high magnesium concentrations. In this chapter, an ATP bioluminescence assay is optimized for highly concentrated salt brines and used to quantify ATP from several brine pools containing varying levels of sodium, magnesium, and chloride.

3.2. Materials and Methods

All reagents were used as obtained except where indicated. ATP Bioluminescence Assay Kits HSII were purchased from Roche Diagnostics (Sigma-Aldrich). TRIS and EDTA were obtained from Sigma-Aldrich. Stock solutions of 10M TRIS and 0.4M EDTA were prepared and used to prepare a 10X Tris-EDTA buffer solution.

3.2.1. Sample Collection

Between 2019 and 2020, samples were collected from several brine pools and surrounding sediment in the South Bay Salt Works (SBSW) salt mine outside of San Diego, California. During the 2019 expedition, I collected a variety of different sample types in sterile 50 mL Falcon tubes. Raw brine samples were taken by submerging a Falcon tube until it was filled. In some cases, samples consisted of salt deposits from the bank of the

Figure 3.3. Samples collected from the 2020 expedition

ponds (surface) or supersaturated salt deposits and small rocks or grains of sand from the bottom of the pond (subsurface). These samples were collected using a garden shovel sterilized with isopropyl alcohol. I also gathered filtered brine samples that were either desalinated using a portable desalinating pump system as well as raw brine 0.2 micron filtrate for extracellular ATP analyses. Samples from the 2020 expedition, example shown in Figure 3.3, were collected by other team members and shipped directly to Georgia Tech in Atlanta Ga within 24 hours to be immediately analyzed for ATP content.

3.2.2. ATP Bioluminescence Assay

Samples were analyzed in a field lab within 48 hours after collection to reduce chances of ATP loss. The bioluminescence assay was conducted according to the Roche ATP Bioluminescence Assay Kit HS II manufacturer's instructions, with some modifications as indicated. Standard curves (Figure 3.4) were generated on a daily basis. Sediment and salt samples were first crushed and homogenized in double layered, sterile

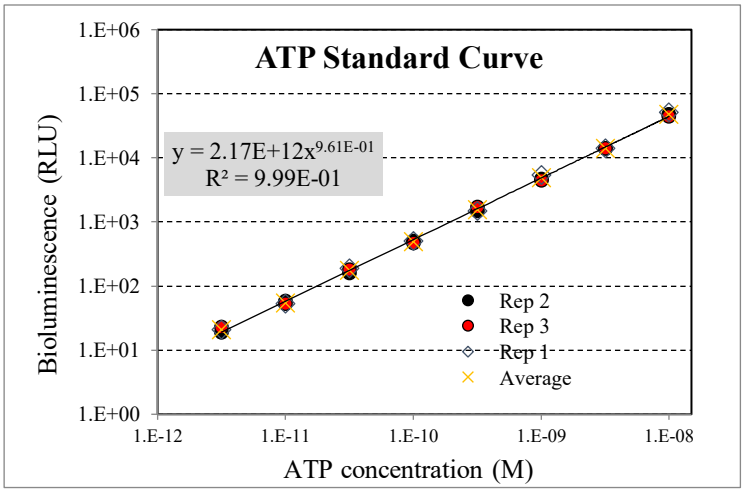


Figure 3.4. ATP standard curve used for quantification calculations. RLU is directly proportional to mass of ATP.

Whirl-Pak bags using a hammer that had been covered with sterilized foil and then divided into triplicate aliquots in 2 mL centrifuge tubes. The samples were then vortexed with a Tris-EDTA buffer (1 mL 100mM TRIS, 4mM EDTA) and submerged in boiled water for 5 minutes to lyse the cells. For the brine samples, 50 mL triplicate aliquots are directly added to the Tris-EDTA buffer before boiling. After cooling back to room temperature, samples were centrifuged for 5 minutes. Exactly 50 µL of supernatant was then added to 50 µL of luciferase reagent immediately before analysis. Bioluminescence was then immediately measured using a Merck HY-LiTE 2 portable luminometer.

3.3. Results and Discussions

ATP content from each site are reported in this section. Assay optimization yielded a high degree of linearity over three orders of magnitude and a lower limit of quantitation of 100-1000 pM depending on salt concentration.

3.3.1. Bioluminescence Assay Optimization

Due to the intense concentrations of salt, the luciferase in the reaction can be deactivated. Therefore a set of dilution ratios (Table 3.1), using Tris EDTA buffer, for each site were determined where a 1:100 dilution is 990 μL TE buffer and 10 μL of brine. The assay is robust to Mg concentrations as high as 3 M, with higher concentrations requiring desalting prior to analysis. Because Mg^{2+} is involved in the luciferase pathway, it is essential to the assay, but at high concentrations is also an inhibitor of the assay. Interestingly, for samples of sites 4 and 5, at no dilution ratio was the high $MgCl_2$ native brine analyzable without utilizing a desalting technique. The following results pertain to the NaCl dominated brine pools, in which native can be analyzed with sufficient dilution.

Table 3.1. Varying concentrations of NaCl and $MgCl_2$ based on site location, Sediment (S) and/or Brine (B) collected during 2019 expedition, and the optimal dilution ratio used in the ATP assay.

Site #	NaCl (M)	$MgCl_2$ (M)	S,B	ATP Assay Dilution Ratios
1	2.7	1.7	S,B	1:00
2	1.2	0.3	S,B	1:10
3	1.4	3.0	S,B	1:100
4	0.14	4.2	S,B	NA
5	0.31	4.7	S,B	NA
6	2.1	2.0	B	1:100
7	2.8	1.6	B	1:100
8	2.3	2.4	S,B	1:100

3.3.2. Filtered vs. Unfiltered Samples

As shown in Figure 3.5, site 2 had the lowest concentration of ATP amongst all other sites. This was unsurprising given that there were brine shrimp and other feeders active in the pool to take up any free ATP. At site 2, I tested to see if ATP concentrations were gradiated by depth in the ponds so I collected water at different depths and determined that there is no significant difference, indicating that the site 2 water is well-mixed. Quantifying free ATP levels, such that is not held within a cell, from filtering brines with

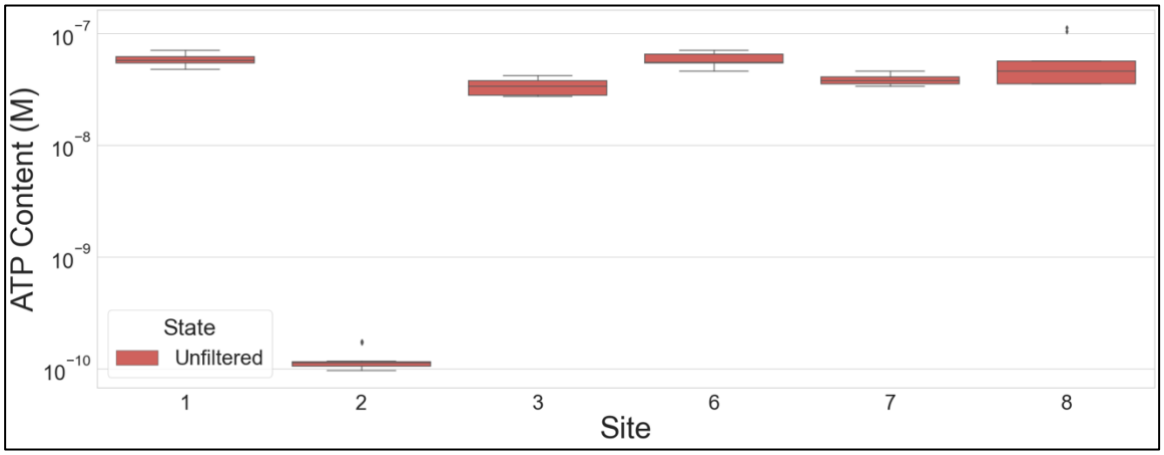


Figure 3.5. Site comparison of ATP content from unfiltered samples, representing both intra and extracellular ATP available.

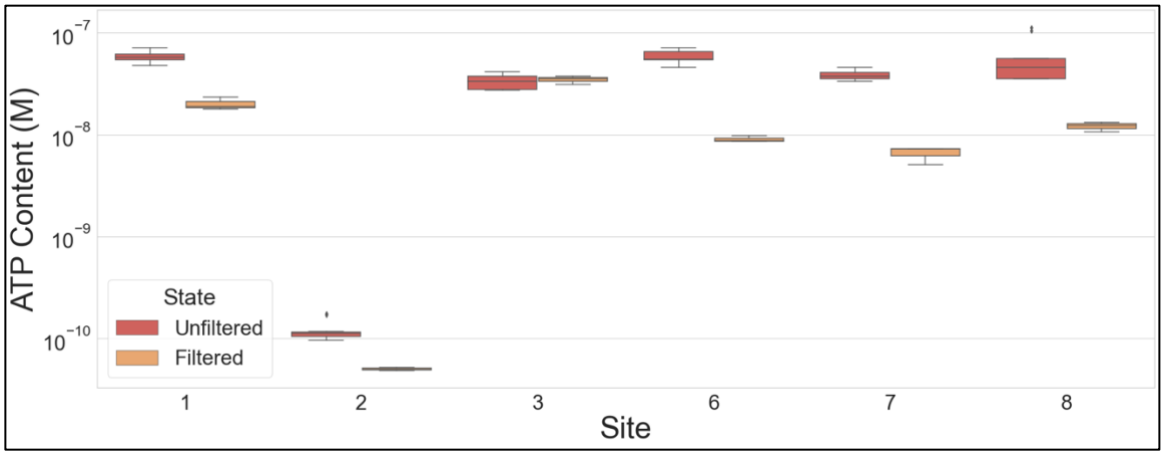


Figure 3.6. Site comparison of ATP content from unfiltered samples, representing both intra and extracellular ATP available, and filtered samples representing only extracellular ATP.

a 0.2 micron filter versus unfiltered samples are shown in Figure 3.6. Filtering the samples removes the cells, leaving the analysis to show only the extracellular ATP information. In the case of site 3, which had higher $MgCl_2$ levels than NaCl, showed that there is more extracellular ATP than cellular ATP. Sites 1, 2, 6, 7, and 8 indicate that the majority of ATP is going to be found within the cells adapted to the high salt concentration habitat.

3.3.3. Conclusions

Magnesium chloride is very soluble in water and therefore can achieve much higher concentrations (>5M), and even lower water activity making life difficult to thrive for even the most salt-tolerant microbes. Although it is expected that brine samples with >3M $MgCl_2$ will be lowest in biomass, samples with this concentration were unable to be analyzed for ATP. Once samples are desalted however, similar dilution techniques can then be applied and yield a more detectable signal but changes the *true* ATP content somewhat significantly. Therefore, further work utilizing extra dilutions and metal leaching buffers will be required to analyze $MgCl_2$ brine in its native state. Pools with higher brine content (> 4 M total NaCl and MgCl2) were found to have higher ATP contents than the pool with lower total brine content (1.5 M total NaCl and MgCl2), indicative of the survival of more organisms to scavenge this molecule in the lower brine content pool and the organic biosignature preservative in higher brine concentrations. Comparison of brine samples filtered through 0.2 um filters and raw samples indicated that all analyzable samples had both extracellular and intracellular ATP, with the higher saline pools having a greater ratio of extracellular to intracellular ATP.

CHAPTER 4

4. CONCLUSIONS

As time has progressed, more and more evidence of potentially habitable worlds outside of Earth itself continued to be discovered and consequently the desire to find signals of past or present life has grown largely within the space exploration community. Some of the biggest questions in astrobiology today are much more specific. *Where* and exactly *how* do we look for signs of extraterrestrial life, and, what are the sources of the building blocks required for life? The aim of this thesis research is to improve sampling strategies for future unmanned missions looking for signs of life, as well as improve an understanding of the preservation of biomarkers in these extreme environments.

Planetary analog research allows scientist to gain a deeper understanding of the limits of life under similar environmental stressors from major astrobiological targets. Using extreme Earth environments as planetary analogs will aid future unmanned exobiology missions by providing more informed, *in situ,* sampling decisions in order to maximize scientific return.

Using a set of nested triangular grids, it is possible to observe how bioactivity potentially spreads or changes at a distance or depth in a variety of extreme analog environments. These triangular grids are useful for observing variations of both small and large scale distributions. Even though ATP, as we know it, is ubiquitous to life terrestrially ATP as a proxy biomarker is a useful way to observe bioactivity in an analog setting.

Because ATP can decay rapidly under normal conditions, it help understand the bioactivity in sample at the time the sample was collected, meaning you can easily keep a record of how concentrations of bioactivity. The ATP bioluminescence assay is a robust method for quantification and can easily be modified to fit high salt content samples, but further work is required to understand effects of high Mg in cellular systems.

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